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The Use of Novel Therapies to Reconstitute Blood Cell Production and Promote Organ Performance, Using Bone Marrow Failure as a Model

PRINCIPAL INVESTIGATOR:
Adrianna Vlachos, MD

CONTRACTING ORGANIZATION:
The Feinstein Institute for Medical Research
350 Community Drive
Manhasset, NY 11030

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Adrianna Vlachos, MD

E-Mail: avlachos@northwell.edu (formerly avlachos@nshs.edu)

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Feinstein Institute for Medical Research
350 Community Drive
Manhasset, NY 11030
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14. ABSTRACT

This study was a Phase I/II study to study the use of L-leucine in subjects with transfusion-dependent Diamond Blackfan anemia, a rare, inherited bone marrow failure syndrome. The study accrued subjects appropriately and closed to accrual in May 2016. In total the study opened to patient accrual in 7/2014 and the last patient received study drug until 2/2017. There were 55 patients consented; 12 screen failures; 43 patients evaluable. There were 21 males; the median age was 10 years 4 months. No untoward side effects were attributable to L-leucine. Two patients had a complete remission of their anemia and 5 patients had a partial remission with elevated reticulocyte counts. Ten of the 22 patients with growth potential and complete data had an average increase of 8%ile in growth velocity, independent of the hematologic response at the end of treatment.

15. SUBJECT TERMS
Diamond Blackfan anemia; leucine; transfusion dependence

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INTRODUCTION: Diamond Blackfan anemia (DBA) is a rare, inherited bone marrow failure syndrome characterized by anemia, congenital anomalies and a predisposition to cancer. Patients usually present during infancy or early childhood, but rarely may present in adulthood. In the majority DBA is due to a mutation in a small or large ribosomal protein (RP) subunit leading to RP haploinsufficiency. Treatments for the anemia of DBA include red cell transfusions (with iron chelation), corticosteroid therapy or stem cell transplantation; however, all are fraught with numerous side effects. One report found one complete erythroid response after the use of the branched chain amino acid L-leucine in 6 select patients. Leucine supplementation has been shown to enhance ribosome biogenesis and mRNA translational efficiency. Mouse and fish models of DBA respond to L-leucine with amelioration of anemia. This is a pilot study to test the feasibility of administering leucine to 50 patients with transfusion-dependent DBA, monitoring for clinical hematologic response and side effects. The primary objectives were to determine the feasibility of administering L-leucine in subjects with DBA who are red cell transfusion-dependent and to determine the efficacy of L-leucine to produce a hematologic and growth response. The secondary objective was to determine the safety profile of L-leucine. This Phase I/II study had an anticipated accrual of 50 subjects in 12 sites. A dose of 700 mg/M2 orally three times per day for 9 months was used. Inclusion criteria included age > 2 years, the diagnosis of DBA and transfusion dependence with adequate kidney and liver function. Response was evaluated at 9 months with Complete Response (CR) defined as no further transfusions required and Hb >9; Partial Response (PR): Hb < 9 gm/dL with an increase in reticulocyte count; and No Response (NR): no change in Hb or reticulocyte count. Growth percentiles were evaluated at baseline and at completion of treatment and the growth velocity change was calculated.

BODY: It took a long time to open this study. Initially the protocol was delayed due to the unanticipated need for an investigational new drug (IND) distinction from the Food and Drug Administration (FDA). The protocol also went through multiple modifications and revisions as required by our local institutional review board (IRB), the Department of Defense (DOD), and the FDA. In addition, the manufacturing company has a shortage of Leucine due to a factory issues so we had to wait before we could obtain the study drug. The local IRB did not approve administration of the product in powder form due to inaccurate measurements with the initially proposed “scoop” method. Thus once purchased the powder also had to be sent to be capsulated.

The study finally opened to accrual in July 2014 and the last subject was enrolled in May 2016 and completed the protocol in February 2017. In total 55 subjects were consented; 12 were screen failures; and, 43 subjects were evaluable. There were 21 males; the median age of all evaluable subjects was 10years 4months. There were 2 severe adverse events reported during this study. No untoward side effects were attributable to L-leucine. Two subjects had a complete remission (CR; subject 05-002 and subject 09-002) and 5 subjects had a partial remission (PR) with elevated reticulocyte counts. Ten of the 22 subjects with growth potential and complete data had an average increase of 8%ile in growth velocity, independent of the hematologic response at the end of treatment.

KEY RESEARCH ACCOMPLISHMENTS:

- The trial of L-leucine in transfusion-dependent subjects was successfully completed.
- This was the first such trial in patients with DBA in multiple institutions in the United States.
- The study drug L-leucine was well-tolerated and deemed safe in this patient population.

- L-leucine resulted in an erythroid response in 16% of subjects.
- L-leucine also resulted in an increase in growth velocity in some subjects.

REPORTABLE OUTCOMES:

Vlachos A, Atsidaftos E, Muir E, Lababidi ML, Alhushki W, Farrar JE, Glader B, Gruner BA, Hartung H, Knoll C, Lowe TW, Nalepa G, Narla A, Panagrahi A, Rogers ZR, Sieff CA, Walkovich K, Lipton JM. Leucine for the Treatment of Transfusion Dependence in Patients with Diamond Blackfan Anemia. *Blood,* 132(Suppl 1), 755. https://doi.org/10.1182/blood-2018-99-113570.

Vlachos A, Atsidaftos E, Muir E, Lababidi ML, Alhushki W, Farrar JE, Glader B, Gruner BA, Hartung H, Knoll C, Lowe TW, Bernstein J, Nalepa G, Narla A, Panagrahi A, Rogers ZR, Sieff CA, Walkovich K, Lipton JM. Leucine for the Treatment of Transfusion Dependence in Patients with Diamond Blackfan Anemia. American Society of Pediatric Hematology/Oncology. May 2019. New Orleans, LA. Invited Oral Presentation in the Hematology Science session.

CONCLUSION:

L-leucine resulted in an erythroid response in 16% of subjects. It was well-tolerated and safe in subjects with DBA and may be used in some patients to achieve independence from chronic transfusion therapy. The use of L-leucine also resulted in an increase in growth velocity in some subjects, irrespective of their hematologic response. Since short stature is a well described complication of DBA the use of L-leucine may be a beneficial drug for all patients. Based on these results, a future study with higher doses of L-leucine should be well-tolerated in this patient population and may lead to even more responses. Additionally patients receiving steroid therapy may demonstrate a similar benefit, leading to decrease or discontinuation of the steroid therapy and thus the steroid side effects.

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APPENDICES:

There are 2 appendices: Table 1 is an enrollment log of all the subjects and Table 2 is a list of all the study sites.

Appendix

Table1. Enrollment Log

Site ID	Subject ID	DOB	Gender	Consent Date	Reconsent if not within 30 days of start of enrollment	Enroll Date	Evaluable? (Y/N)	Reason unevaluable	Date Completed/ Unevaluable	Treatment Period Started	Treatment Period Ended	Withdrawn (Y/N)
01	01-001-KG	3/12/2010	F	17-Jun-13	N/A	17-Jun-13	Y	N/A	25-Sep-13	15-Jul-13	13-Aug-13	Y
01	01-002-JB	12/7/2006	M	17-Jun-13	N/A	27-Jun-13	Y	N/A	26-Mar-14	27-Jun-13	26-Mar-14	N
01	01-003-LB	10/13/1986	F	17-Jun-13	N/A	28-Jun-13	Y	N/A	24-Mar-14	8-Jul-13	24-Mar-14	N
01	01-004-SK	6/12/1967	F	22-Jun-13	N/A	5-Jul-13	Y	N/A	25-Mar-14	12-Jul-13	25-Mar-14	N
01	01-005-BH	12/21/2010	M	26-Jun-13	N/A	28-Jun-13	Y	N/A	31-Mar-14	10-Jul-13	31-Mar-14	N
01	01-006-OK	12/12/2007	F	28-Jun-13	N/A	13-Aug-13	Y	N/A	19-Sep-13	21-Aug-13	17-Sep-13	Y
01	01-007-AL	6/12/2004	F	16-Jul-13	N/A	16-Jul-13	Y	N/A	30-May-14	12-Sep-13	30-May-14	N
01	01-008-MB	9/6/1998	F	19-Jul-13	N/A	1-Jan-00	Y	N/A	1-Jun-14	16-Aug-13	1-Jun-14	N
01	01-009-AM	12/7/1998	M	29-Jul-13	N/A	29-Jul-13	Y	N/A	12-May-14	16-Aug-13	12-May-14	N
01	01-010-MM	12/7/1998	M	29-Jul-13	N/A	29-Jul-13	Y	N/A	12-May-14	16-Aug-13	12-May-14	N
01	01-011-TC	6/11/1983	M	2-Aug-13	N/A	N/A	N	Screen Failure	N/A	N/A	N/A	Y
01	01-012-ER	6/18/1997	F	5-Nov-13	N/A	12-Nov-13	Y	N/A	19-Aug-14	21-Nov-13	19-Aug-14	N
01	01-013-OFG	10/10/2007	M	18-Dec-13	N/A	18-Dec-13	N	Parental Decision	N/A	N/A	N/A	Y
01	01-014_RJK	8/30/1996	M	19-Dec-13	N/A	N/A	N	Screen Failure	N/A	N/A	N/A	Y
01	01-015-LP	9/19/2003	M	30-Dec-13	N/A	N/A	N	Screen Failure	N/A	N/A	N/A	Y
01	01-016-OD	1/18/2008	F	9-Feb-14	N/A	12-Mar-14	Y	N/A	15-Dec-14	21-Mar-14	15-Dec-14	N
01	01-017-	1/18/2008	F	9-Feb-14	N/A	12-Mar-14	Y	N/A	15-Dec-14	21-Mar-14	15-Dec-14	N
01	01-018-KG	9/19/2003	F	5-Sep-14	Withdrawn	Withdrawn	N	Withdrew	N/A	N/A	N/A	Y
02	02-001-PV	11/26/1997	M	7-Feb-14	N/A	11-Mar-14	Y	N/A	2-Dec-14	11-Mar-14	2-Dec-14	N
02	02-002-ML	12/18/1997	F	7-Feb-14	N/A	N/A	N	Screen Failure	20-Mar-14	N/A	N/A	Y
02	02-005-SG	8/21/2009	F	5-May-14	18-Sep-14	8-Oct-14	Y	N/A	1-Sep-15	18-Nov-14	1-Sep-15	N
02	02-007-SM	11/22/1964	F	12-May-14	N/A	N/A	N	Screen Failure	15-Oct-14	N/A	N/A	Y
02	02-003-CS	9/26/1998	M	19-May-14	24-Sep-14	15-Oct-14	Y	N/A	28-Jul-15	28-Oct-14	28-Jul-15	N
02	02-004-SO	8/22/1997	F	21-May-14	20-Sep-14	4-Oct-14	Y	N/A	21-Apr-15	24-Oct-14	21-Apr-15	Y
02	02-006-SJ	4/18/2008	M	21-Jun-14	N/A	N/A	N	Screen Failure	15-Oct-14	N/A	N/A	Y
02	02-008-BS	8/30/2007	M	11-Jun-15	N/A	10-Jul-15	Y	N/A	1-Mar-16	30-Jul-15	1-May-16	N
03	03-001-ME	9/13/2007	M	26-Mar-14	N/A	3-Apr-14	Y	N/A	6-Jan-15	17-Apr-14	6-Jan-15	N
03	03-002-SM	12/19/1983	M	21-Apr-14	N/A	28-Apr-14	Y	N/A	2-Feb-15	19-May-14	2-Feb-15	N
03	03-003-DW	12/20/1982	M	31-Oct-14	N/A	13-Nov-14	Y	N/A	6-Jan-15	3-Dec-14	6-Jan-15	N
04	04-001-LZ	3/30/2007	M	3-Apr-14	N/A	4-Apr-14	Y	N/A	11-Dec-14	10-Apr-14	11-Dec-14	N
04	04-002-JIS	2/2/2005	F	9-May-14	N/A	16-May-14	Y	N/A	19-Jan-15	13-May-14	19-Jan-15	N
05	05-001-JS	11/21/1999	M	28-Apr-14	N/A	N/A	N	Screen Failure	N/A	N/A	N/A	Y
05	05-002-DH	2/27/1998	M	9-May-14	N/A	13-May-14	Y	N/A	30-Jan-15	23-May-14	30-Jan-15	N
05	05-003-CC	12/31/2004	M	6-Apr-15	N/A	4-May-15	Y	N/A	26-Jan-16	28-May-15	26-Jan-16	N
05	05-004-AM	8/11/2012	F	22-Apr-16	N/A	20-May-16	y	N/A	4-Oct-16	1-Jun-16	4-Oct-16	Y
06	06-001-BF	11/15/1970	M	1-Jul-14	N/A	11-Jul-14	Y	N/A	7-Apr-15	22-Jul-14	7-Apr-15	N
06	06-002-MU	3/26/1971	F	5-Aug-14	N/A	3-Sep-14	Y	N/A	25-Jun-15	23-Sep-14	25-Jun-15	N
06	06-003-AU	5/16/2008	M	5-Aug-14	N/A	3-Sep-14	Y	N/A	16-Jun-15	23-Sep-14	16-Jun-15	N
06	06-004-QB	11/5/2012	F	9-Apr-15	N/A	4-May-15	Y	N/A	29-Jan-16	1-Jun-15	29-Jan-16	N
07	07-001-SJ	11/21/2007	F	24-Jun-14	N/A	27-Jun-14	Y	N/A	31-Mar-15	1-Jul-14	31-Mar-15	N
07	07-002-LK	12/30/1991	F	7-Jul-15	N/A	23-Jul-15	Y	N/A	26-Jan-16	28-Jul-15	26-Jan-16	Y
07	07-003-ER	9/16/2013	F	2-Feb-16	N/A	16-Feb-16	Y	N/A	15-Nov-16	1-Mar-16	15-Nov-16	N
08	08-001-MJ	7/28/2007	F	12-Aug-14	N/A	8-Sep-14	Y	N/A	7-Jul-15	3-Oct-14	7-Jul-15	N
08	08-002-AF	5/25/2004	F	25-Nov-14	N/A	22-Dec-14	Y	N/A	19-Oct-15	15-Jan-15	19-Oct-15	N
08	08-003-AA	2/22/2011	F	5-Jan-15	N/A	4-Feb-15	Y	N/A	15-Dec-15	20-Feb-15	15-Dec-15	N
08	08-004-AE	6/6/2001	M	16-Sep-15	N/A	8-Oct-15	Y	N/A	7-Jul-16	13-Oct-15	7-Jul-16	N
09	09-001	7/3/2003	M	2-Sep-14	N/A	12-Sep-14	Y	N/A	5-Jun-15	17-Sep-14	5-Jun-15	N
09	09-002-MT	7/14/2006	M	31-Aug-15	N/A	28-Sep-15	Y	N/A	6/13/2016	6-Oct-15	13-Jun-16	N
09	09-003-SM	1/19/1991	M	21-Apr-16	N/A	5/16/2016	y	N/A	6-Mar-17	27-May-16	6-Mar-17	N
09	09-004-PM	9/7/1994	M	21-Apr-16	N/A	5/16/2016	y	N/A	3-Mar-17	27-May-16	3-Mar-17	N
11	11-001-DW	12/28/2000	F	19-Sep-14	N/A	10-Oct-14	Y	N/A	17-Jul-15	28-Oct-14	17-Jul-15	N
11	11-002-JW	8/16/1980	M	31-Jul-15	N/A	N/A	N	Screen Failure	21-Sep-15	N/A	N/A	Y
12	12-001-CA	2/8/2008	M	4-Nov-14	N/A	25-Nov-14	N	Withdrew	19-Dec-14	N/A	N/A	Y
12	12-002-BM	6/12/2011	M	25-Nov-14	N/A	N/A	N	Screen Failure	19-Dec-14	N/A	N/A	Y
12	12-003-CA	2/8/2008	M	6-Jul-15	N/A	23-Jul-15	Y	N/A	12-May-16	4-Sep-15	12-May-16	N

Table 2. Site List

Site	DOD reference #	Local IRB Protocol #	Site PI	Site Coordinator	Coordinator Contact	IRB Approval Date	DOD Approval Date	Site Initiation Vis	# of Pts Ap
01-Feinstein Institute for Medical Research (Manhasset, NY)	A-16175.a	IRB 12-375B	Adrianna Vlachos, MD	Eva Atsidaftos	eatsidaf@nshs.edu	10-Jun-13	11-Jun-13		20
02-Stanford University (Stanford, CA)	A-16175.b	IRB-27253	Bertil Glader, MD, PhD	Heather Hilmoe	hhilmoe@stanford.edu	6-Aug-13	20-Dec-13	30-Jan-14	6
03-Children's Hospital of Philadelphia (Philadelphia, PA)	A-16175.c	IRB-13-010091	Helge Hartung, MD	Beverly Paul RN	PAUL@email.chop.edu	30-Jan-14	20-Mar-14	27-Jan-14	12
04-University of Michigan Health System (Ann Arbor, MI)	A-16175.d	HUM00080618	Kelly Walkovich, MD	Ashley Shaver	akshaver@med.umich.edu	28-Jan-14	25-Mar-14	31-Mar-14	5
05-University of Texas Southwestern (Dallas, TX)	A-16175.e	STU032013-081	Zora R. Rogers, MD	Leah Adix	LEAH.ADIX@childrens.com	25-Nov-13	25-Mar-14	22-Apr-14	10
06-Children's Hospital Boston (Boston, MA)	A-16175.f	IRB-P00009112	Colin A. Sieff, MD	Krystle Benedict	Krystle.Benedict@childrens.harvard.edu	14-Oct-13	3-Apr-14	1-May-14	10
07- Riley Hospital for Children (Indianapolis, IN)	A-16175.g	IRB-1312051728 | N	Grzegorz Nalepa, MD	Shannon Maraldo, CCRP	saranjo@iu.edu	11-Feb-14	13-Jun-14	23-Jun-14	3
08-Phoenix Children's Hospital (Phoenix, AZ)	A-16175.h	IRB-13-105	Christine Knoll, MD	Erica Olson, RN	eolson1@phoenixchildrens.com	19-Dec-13	13-Jun-14	23-Jun-14	3
09-University of Missouri (Columbia, MO)	A-16175.i	IRB-1209323	Thomas W. Lowe, MD	Kim Ebersol	ebersolk@health.missouri.edu	22-Jan-14	22-May-14	29-May-14	2
10-Memorial Health University Medical Center (Savannah, GA)	withdrawn	closed	Martin Johnston, MD	Yvetta Lee		4-Mar-14	withdrawn		
11-University of Louisville (Louisville, KY)	A-16175.k	IRB-14.0268	Arun Panagrahi, MD	April Loveall/ Kayla Bowling	april.loveall@nortonhealthcare.org	17-Apr-14	5-Aug-14	27-Aug-14	3
12-Children's Specialty Center of Nevada (Las Vegas, NV)	A-16175.j	WIRB -20140525	Jonathan Bernstein, MD	Daniel Crosier	dcrosier@cure4thekids.org	8-Apr-14	5-Sep-14	10-Sep-14	2
13- University of Arkansas (Little Rock, AK)	A-16175-l	IRB-203237	Jason Farrar, MD	Jason Farrar, MD	JEFarrar@uams.edu	14-Apr-15	3-Nov-15	14-Apr-16	2